

EXHIBIT NO.

4

Human rhinoviruses and enteroviruses in influenza-like illness in Latin America

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Background

Human rhinoviruses (HRVs) belong to the *Picornaviridae* family with high similarity to human enteroviruses (HEVs). Limited data is available from Latin America regarding the clinical presentation and strains of these viruses in respiratory disease.

Methods

We collected nasopharyngeal swabs at clinics located in eight Latin American countries from 3,375 subjects aged 25 years or younger who presented with influenza-like illness.

Results

Our subjects had a median age of 3 years and a 1.2:1.0 male:female ratio. HRV was identified in 16% and HEV was identified in 3%. HRVs accounted for a higher frequency of isolates in those of younger age, in particular children < 1 years old. HRV-C accounted for 38% of all HRVs detected. Phylogenetic analysis revealed a high proportion of recombinant strains between HRV-A/HRV-C and between HEV-A/HEV-B. In addition, both EV-D68 and EV-A71 were identified.

Conclusions

In Latin America as in other regions, HRVs and HEVs account for a substantial proportion of respiratory viruses identified in young people with ILI, a finding that provides additional support for the development of pharmaceuticals and vaccines targeting these pathogens.

HRV and HEV in Central and South America

We collected 3,375 nasopharyngeal swabs from subjects with ILI symptoms from eight countries throughout Central and South America. We performed direct RT-PCR for HRVs and HEVs and sequenced all positive samples ($n = 632$) (Figure 1). Our subjects had a median age of 3 years, ranging from less than 1 month to 25 years, an interquartile range of 1 to 8 years, and a male/female ratio of 1.2:1.

Overall, HRVs and HEVs were identified in 16% (548 samples) and 3% (84 samples) of the ILI cases, respectively. Among the HRVs, HRV-A was the most represented species (9% of ILI cases), followed by HRV-C (6%) and HRV-B (1%). Although the number of ILI samples collected among countries varied considerably (Figure 2, lower panel) we found no statistically significant geographic differences in the proportions of HEV, HRV-A, HRV-B, and HRV-C.

Coxsackieviruses comprised the majority of the HEV group (65% of the HEVs identified), showing a variety of types: 9 for coxsackievirus A and 5 for coxsackievirus B (Figure 3). In addition, we detected multiple HEV serotypes, including EV-D68, EV-C99, EV-C104, EV-C109, and EV-B110. We also identified EV-A71 in two participants, both from the department of Tumbes, Peru, although from different cities. The first subject, a one year-old girl from the city of Tumbes (same name as the department), presented on April 27, 2011, with fever, rhinorrhea, cough, and erythema on pharyngeal examination. The second, a two year-old girl from the city of Zarumilla, presented on May 10, 2011, with fever, malaise, rhinorrhea, cough, and weight loss. Neither subject had rash, gastrointestinal manifestations, convulsions, change in consciousness, or other neurological deficits. Lastly, two polioviruses were detected and were related to the Sabin-1 polio vaccine strain. (my emphasis)